In this work, we focused on the investigation of the influence of radiofrequency (RF) electromagnetic waves upon the red cells from animal blood, including the combined action of RF waves and magnetic fluid. The hemolysis phenomenon was investigated on the basis of light extinction at the wavelengths of 540 nm and 575 nm. In all exposed samples, the hemoglobin level is higher than in the control-unexposed samples, revealing the intensification of hemolysis phenomenon following the non-thermal RF electromagnetic exposure, for 2 and respectively 14 hours. The red blood cells released higher concentration of hemoglobin following the electromagnetic exposure and/or ferrofluid addition, suggesting the significant amplification of the RF waves damaging effect on the erythrocytes’ membrane stability in the presence of magnetite nanoparticles.

Key words: electromagnetic exposure, magnetic fluid, animal blood, spectral measurements.

1. INTRODUCTION

The experimental investigation presented below was focused on the influence of both radiofrequency electromagnetic waves and biocompatible magnetic fluid, upon the bovine red blood cells. The motivation of this paper’s objectives is based on the followings.

(i) There is a rich literature in the field of erythrocyte hemolysis, induced by radiofrequency tools utilization in the treatment of carcinoma through laparoscopic RF ablation [1–4], as well as in the scientific reports related to the experiments dedicated to controlled electromagnetic exposure to RF waves of in vitro red blood cells [5–8]. The wide extent of industrial and domestic utilization of electromagnetic fields, in the range of microwaves and radiofrequency waves, triggered a
significantly large development of experimental and epidemiological studies [9–11]. Short review on the blood response to magnetic and electromagnetic fields [11] reveals some interesting aspects, such as: diminution of erythrocytes sedimentation speed, enhancement of fagocitary activity of leucocytes, shift of globulin fractions etc. More recently, it was reported by D. Ciorba and V. Morariu [12], that exposure to zero-magnetic field resulted in hemolysis intensification. However, we failed in finding data on the biological effects of combined action of RF exposure and magnetic nanoparticle biomedical administration.

(ii) The utilization of magnetic fluids in diagnosis and therapy, as modern tools in contemporary medical practice, determined various researches regarding the interaction of blood cells with magnetic nanoparticles [13]. Biomedical use of biocompatible magnetic fluids includes mainly: magnetically controlled targeted micro-carrier systems, contrast agents in Magnetic Resonance Imagery, magnetic cell sorting and separation, magnetic DNA purification system, hyperthermia induced by agents for cancer therapy, radio-immunity analysis, etc. [14–20]. Since in most of these applications, the blood cells interaction with the magnetic nanoparticles is unavoidable, the response of erythrocytes membranes to low levels of magnetic nanoparticle concentrations is of great interest. Generally, literature reports present positive results in pointing up the magnetic fluid’s in vitro biocompatibility with the red blood cells, the cytotoxicity tests proving no hemolysis activity [14–16]. There is little literature mentioning the hemolytic effects of magnetic nanoparticles. For instance, the accelerated hemolysis was evidenced in the frame of experimental study involving both visible radiation and magnetic nanoparticle addition to in vitro red blood cells [21].

This is why the experiment carried out by us was focused on the combined effect of radiofrequency wave irradiation and magnetic nanoparticle addition, since such situation actually occurs during ferrofluid based tumor hyperthermia or during ferrofluid utilization in NMR imagery, in biomedical research and clinical diagnosis and therapy.

2. MATERIALS AND METHODS

Biologic material consists in whole animal blood – bovine (provided by the Faculty of Veterinary Medicine of Iasi) – on anticoagulant of ACD type. Blood samples (approximately 7.0 ml) were designed as experimental variants:

- exposed blood – to radiofrequency waves in the absence of magnetic fluid;
- treated blood – with magnetic fluid in the absence of electromagnetic exposure;
- treated blood – with magnetic fluid and exposed to radiofrequency waves;
- control sample – neither electromagnetically exposed nor treated with magnetic fluid.
The biocompatible magnetic fluid was consistent with colloidal magnetic suspension yielded by magnetite cores stabilization with TMA (tetramethyl ammonium hydroxide) [15] and suspension in water (as carrier fluid) [22]. Two concentrations of magnetic fluid were tested: \( C_1 = 0.5\% \) and \( C_2 = 3.0\% \).

Electromagnetic exposure The exposure device was a transverse electromagnetic cell, designed to deliver low power density of 0.6 mW/cm\(^2\), at a frequency of 418 MHz [9]. The TEM device (Fig. 1), built in aluminum, has adequate dimensions calculated to assure the characteristic impedance \( Z_0 \) of 50 \( \Omega \). The transverse propagation mode is the dominant one. The blood samples were exposed to the TEM cell for various exposure times (continuous wave exposure): 2 and 14 hours.

![Fig. 1. – Electromagnetic exposure system.](image)

For each exposed sample, it was considered a control sample (neither exposed to RF waves nor treated with magnetic fluid), placed in identical light and temperature conditions, but completely isolated from the action of RF waves coming from the exposure device.

Blood analysis Following the electromagnetic exposure, all blood samples were incubated at 37.5\(^\circ\)C ± 0.1\(^\circ\)C within a water bath, for 30 minutes and further centrifuged for 5 minutes by 3,000 cycles/min. The supernatant was withdrawn and submitted to the spectrophotometric investigation. Meterteck SP170-Plus-type for visible range spectrophotometer, provided with quartz cells of 1.0 cm width, was used to record the hemoglobin spectra (450–600 nm) in the blood samples. Hemolysis extent was assessed using the formula:

\[
H.E. = \frac{A_s - A_c}{A_c},
\]

where \( A_s \) is the absorbance of the exposed sample at 540 nm and \( A_c \) – the absorbance of the control sample at the same wavelength.

The absorbance ratio at the wavelengths of 540 nm and 575 nm was also calculated, aiming to quantitatively express the changes induced in the blood samples compared to the control:
where \( A_{540\text{nm}} \) is the absorbance of the exposed sample at 540 nm, \( A_{575\text{nm}} \) is the absorbance of the exposed sample at 575 nm.

**Statistic significance** of the spectrophotometric investigation was ensured by working with three replies of every exposed sample or unexposed controls. Average values and standard deviations were utilized for the graphic representations.

3. RESULTS AND DISCUSSIONS

The hemoglobin spectra recorded in the blood supernatants are similar to those published in the literature (Fig. 2).

![Absorption spectra of blood supernatant of the control sample and of the samples exposed for 2 hours to RF radiation, respectively, for 14 hours to RF radiation.](image)

In all exposed samples, the hemoglobin level is higher than the one in the control sample, revealing the intensification of hemolysis phenomenon following the RF electromagnetic exposure and, particularly, following the addition of magnetic fluid (Fig. 3). The influence of the exposure time did not lead to important differences, as it can be seen in Fig. 2.

The electromagnetic exposure of blood samples in the presence of magnetic fluid resulted in the increase of light extinction, especially in the range from 500 nm to 600 nm, but it is linearly dependent on the exposure time (the highest value it is obtained at 2 hours electromagnetic exposure).
The effect of the RF exposure upon the hemolysis extent. The exposure of blood samples to the RF waves, in the absence of magnetic fluid, resulted in the increase of hemolysis extent as shown in Fig. 4.
The absorption spectrum of hemoglobin was not modified – there were no band shifts; however, the intensity of the two peaks (the absorbance) has increased with the exposure time, especially at 540 nm, being used in the calculation of the hemolysis extent; in other words, the hemolysis phenomenon got more intense in the exposed blood samples for longer exposure times (14 hours); monotonous increase of the hemolysis extent to the RF exposure time was found.

**The effect of the ferrofluid upon the hemolysis extent** Linear approach of the hemolysis extent, and also of the extinction maxima ratio to the ferrofluid concentration, was revealed. In the absence of RF waves exposure, the hemolysis extent significantly increased with higher concentration of magnetic fluid (Fig. 5), following a theoretical line, with a correlation coefficient higher than 0.95.

![Graph showing linear approach of hemolysis extent to ferrofluid concentration](image)

**Fig. 5.** – Hemolysis extent in blood samples for different ferrofluid concentrations without electromagnetic exposure – linear approach.

Thus, the red blood cells release higher concentration of hemoglobin following the interaction with the magnetic nanoparticles, suggesting undesired side effects of the traces of magnetic fluid remaining for some time in the patient’s blood, when used in medicine applications.

**The combined effect of both RF exposure and ferrofluid addition on the hemolysis extent** The graph presented in Fig. 6 reveals the hemolysis extents for different exposure times to radiofrequency waves in blood samples with various concentrations of magnetic fluid.

Two times higher hemolysis extent was obtained for 3.0% ferrofluid in blood samples compared to the 0.5% concentration of ferrofluid for the same RF exposure times: 2 h and respectively, 14 h, i.e. more than the sum of the hemolytic effects of separate application of either RF exposure or magnetic nanoparticles.
Radiofrequency electromagnetic wave and paramagnetic particles effects on the red blood cells

Unlike the previous case, in the samples containing magnetic fluid, a decrease of the hemolysis phenomenon seems to occur at higher exposure time (for both tested concentrations) comparatively to the short exposure time.

**The combined effect of the RF exposure and ferrofluid addition on the extinction maxima ratio.** As in the case of the hemolysis extent, the ratio of the two light extinctions in the visible range of the hemoglobin spectrum is always higher in the blood experimental unexposed variants in the presence of magnetic fluid than in the blood control sample (Fig. 7).

It can be noticed that there is a very good linear correlation between the absorbance ratio and the concentration of ferrofluid (correlation coefficient $R = 0.9636$),
similar with the case of the hemolysis extent, where the correlation coefficient R = 0.9895 has been obtained.

One can see, that with the increase of the concentration of the magnetic fluid (Fig. 8), the absorbance ratio at the two wavelengths, in the visible range of the haemoglobin spectrum, is increasing along with each duration of electromagnetic exposure. As in the previous case (non-exposed samples to RF waves), the most significant increase corresponds with the blood samples exposed for 2 hours (12%). Simultaneously, for each concentration of ferrofluid, the absorbance maxima ratio increases for the electromagnetic exposed blood samples – the highest value corresponds to the 2 hours exposure. The overlapped action of the RF waves and magnetic fluid resulted in the increase of the hemolysis extent and of the two maxima ratio, possibly due to the paramagnetism of the iron ions; they are able to absorb energy from the electromagnetic field of RF frequency and, consequently, can damage an increased number of cells, enhancing the concentration of released hemoglobin.

![Fig. 8](image.png)

The iron ions of the ferrophase from the magnetic fluid could affect the relative intensity of the two electronic absorption bands studied inhere, probably due to the fact that these transitions are located at the heme level – the iron ions being also involved. The interaction between the iron ions from the ferrofluid nanoparticles and the heme structure seems to be able to modify the transition probabilities corresponding to the main two absorption bands of hemoglobin from the visible range (the iron-iron interaction between hemoglobin and magnetite nanoparticles was evidenced by means of Raman vibration spectra in [23]).
4. CONCLUSIONS

The amplification of the electromagnetic exposure hemolytic effect in the presence of magnetic nanoparticles, provided in colloidal form by means of magnetic fluid addition, was revealed.

The hemolysis extent was significantly higher than the sum of the values corresponding to the RF radiation exposure and respectively, to magnetic nanoparticles impact. Hemoglobin release was more intense in the case of magnetic nanoparticle addition (in the absence of the RF radiation exposure) than in the case of RF wave exposure (in the absence of the magnetic nanoparticles).

The biocompatibility of magnetite-TMA magnetic fluid seems to be strongly dependent on its concentration in the blood samples as well on the electromagnetic conditions.

REFERENCES


